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(54) Title: METHODS FOR TREATING GLAUCOMA

(57) Abstract: This invention provides a method for treating a subject with glaucoma comprising the steps of administrating a compound or composition which antagonizes, inhibits, inactives, reduces, suppresses, and/or limits the release, synthesis, or production

METHODS FOR TREATING GLAUCOMA

FIELD OF THE INVENTION

This invention provides a method for treating a subject with glaucoma comprising the steps of administrating a compound or composition which antagonize, inhibits, inactivates, reduce, suppresses, antagonizes, and/or limits the release, synthesis, or production from cells of TNF-α thereby treating the subject with glaucoma.

BACKGROUND OF THE INVENTION

The cytokine known as tumor necrosis factor (TNF or TNF–α) is structurally related to lymphotoxin. They have about 40 percent amino acid sequence homology (Old, Nature 330:602-603, 1987). These cytokines are released by macrophages, monocytes and natural killer cells and play a role in inflammatory and immunological events. The two cytokines cause a broad spectrum of effects both in vitro and in vivo, including: (i) vascular thrombosis and tumor necrosis; (ii) inflammation; (iii) activation of macrophages and neutrophils; (iv) leukocytosis; (v) apoptosis; and (vi) shock. TNF has been associated with a variety of disease states including various forms of cancer, arthritis, psoriasis, endotoxic shock, sepsis, autoimmune diseases, infections, obesity, and cachexia. TNF appears to play a role in the three factors contributing to body weight control: intake, expenditure, and storage of energy (Rothwell, Int. J. Obesity 17:S98-S101, 1993).

Histopathologic studies of the glaucomatous optic nerve head in primary open angle glaucoma (POAG) reveal astroglial activation and tissue remodeling, which accompanies neuronal damage. As a part of tissue remodeling, backward bowing and disorganization of the laminar cribriform plates are common characteristics of glaucomatous eyes with either normal or high intraocular pressure.[1] These histologic changes are accompanied by the upregulation of extracellular matrix components including collagen and proteoglycan, and adhesion molecules by optic nerve head astrocytes in glaucomatous eyes.[2-6] The astroglial activation seen in glaucomatous optic nerve heads likely represents an attempt to limit the extent of the injury and promote the tissue repair process. However, despite the astroglial activation, there is limited

deposition of extracellular matrix in glaucomatous optic nerve atrophy, which does not retain characteristics of scar tissue formation.[7,8] This suggests that there are diverse cellular responses to the initial event or subsequent tissue injury, which preferentially results in tissue degradation.

In addition, reactive astrocytes following neuronal injury produce various neurotrophic factors and cytokines including TNF-α. [14] which play a critical role in the regulation of the synthesis of MMPs.[15-17] Furthermore, the release of TNF-α from its membrane-bound precursor is a MMPs-dependent process.[18] Matrix metalloproteinases (MMPs) are proteolytic enzymes that degrade components of extracellular matrix. Increased secretion of MMPs by activated glial cells have been implicated in various extracellular matrix remodeling events that occur during normal development and in a number of pathologies including atherosclerosis, arthritis, tumor growth, metastasis and glaucoma.[9-13] .

TNF-α is a potent immuno-mediator and pro-inflammatory cytokine that is rapidly upregulated in the brain after injury. It is also known as an inducer of apoptotic cell death via TNF-α receptor-1 occupancy (Hsu H, Xiong J, Goeddel DV. The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell.* 1995;81:495-504.).

Open angle glaucoma (OAG) the second leading cause of irreversible blindness in the United States, comprises 2 major syndromes: primary open angle glaucoma (POAG) and normal pressure glaucoma (NPG). POAG is a disease generally characterized by a clinical triad which consists of 1) elevated intraocular pressure (IOP); 2) the appearance of optic atrophy presumably resulting from elevated IOP; and 3) a progressive loss of peripheral visual sensitivity in the early stages of the disease, which may ultimately progress and impair central visual acuity. (Quigley, HA: Open angle glaucoma. New Engl J Med 1993; 328:1097-1106.) Studies have indicated, however, that a surprisingly high percentage of patients with OAG have findings identical to those in POAG but with a singular exception; namely, that the IOP has never been demonstrated to be elevated. Several large population-based studies have documented the high prevalence of this form of glaucoma, often called "low tension glaucoma" (but more accurately

called "normal pressure glaucoma") (NPG). The most conservative of these estimates place the percentage of glaucoma that occurs in the presence of "normal" IOP at approximately 20-30% (Sommer A. Intraocular pressure and glaucoma. *Am J Ophthalmol.* 1989;107:186-188. and Sommer A. Doyne Lecture, Glaucoma: Facts and Fancies. *Eye* 1996;10:295-301.)

In addition to the most common forms of glaucoma described above, there are secondary and closed angle forms of glaucoma which typically result in elevated intraocular pressure due to a variety of mechanisms. In virtually all these other forms of glaucoma, elevated eye pressure is found, and a characteristic optic neuropathy similar to that found in OAG ensues. If untreated, elevated intraocular pressure in these glaucomas invariably leads to visual loss and eventual blindness. In many forms of glaucoma, including those with normal intraocular pressure, lowering of intraocular pressure often fails to halt the progression of the disease. Comparison of glaucomatous progression between untreated patients with normal-tension glaucoma and patients with therapeutically reduced intraocular pressures. Collaborative Normal-Tension Glaucoma Study Group. Am J Ophthalmol. 1998 Oct;126(4):487-97.)

During development and maintenance of the nervous system there exists a complex interdependency between neurons and glial cells. The glial cells maintain normal functioning of the nervous system both by controlling the extracellular environment and by supplying metabolites and growth factors. After damage to the central nervous system, glial cells are thought to support neural growth and metabolism and to scavenge agents toxic to neurons. However, recent evidence challenges the view that glial cells are purely neuroprotective and rather suggests that they could participate in damaging neurons. For example, following focal cerebral ischemia or during the course of neurodegenerative diseases or trauma, reactive astrocytes as well as microglia within the central nervous system produce cytokines, reactive oxygen species and nitric oxide (NO), which are implicated as mediators of tissue injury.

SUMMARY OF THE INVENTION

As provided herein, the invention provides a compound or composition containing an agent or molecule which antagonize, inhibits, inactivates, reduce, suppresses, antagonizes, and/or limits the release, synthesis, or production from cells of TNF-α. Such a composition is beneficial for the treatment of glaucoma.

This invention provides a method for treating a subject with glaucoma comprising the steps of administrating a compound, agent or composition containing an agent, compound, or molecule, including analogs, isomers, homologues, fragments or variants thereof, which antagonize, inhibits, inactivates, reduce, suppresses, antagonizes, and/or limits the release, synthesis, or production from cells of TNF-α thereby treating the subject with glaucoma.

In one embodiment, the agent, compound, or molecule suppresses the level or production of TNF-α. In another embodiment, the agent, compound, or molecule inhibits the production of TNF-α.

In another embodiment, the agent, compound, or molecule limits the synthesis or release of TNF-α from cells. In another embodiment, the compound is thalidomide. In another embodiment, the compound is a selective cytokine inhibitor. In another embodiment, the inhibitor is rolipram, phosphodiesterase 4 inhibitor, or p38 kinase.

In another embodiment, the agent, compound, or molecule inactivates circulating TNF-α. In another embodiment, the molecule is anti- TNF-α antibody. In another embodiment, the molecule is infliximab. In another embodiment, the molecule is recombinant TNF-α soluble receptors. In another embodiment, the molecule is etanercept.

This invention provides a TNF reducer which is hydrazine sulfate, pentoxifylline, ketotifen, tenidap, vesnarinone, cyclosporine, peptide T, sulfasalazine, thorazine, antioxidants, corticosteroids, marijuana, glycyrrhizin, sho-saiko-to, L-carnitine, hyperthermia, or hyperbaric oxygen therapy.

Lastly, this invention provides a method of assaying a subjects serum level of TNF alpha as an indicator for treatment with TNF inhibitors. The assay measures the level of several cytokines in the serum of the subject such as interleukion 10 and interferon gamma.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Immunoperoxidase staining for TNF-α in the human optic nerve head. There was faint immunostaining of a few glial cells around the nerve bundles and blood vessels (v) in the prelaminar region of the control optic nerve head. (A). However, the intensity of the immunostaining and the number of stained glial cells were greater in the optic nerve heads from patients with primary open angle glaucoma (B) or normal pressure glaucoma (C) (gc, glial column; nb, nerve bundles; cs, cavernous spaces) (Chromagen, DAB; Nuclear counterstain with Mayer's hematoxylin; original magnification X 100).

Figure 2. Immunoperoxidase staining for TNF-α receptor-1 in the human optic nerve head. Faint immunostaining of the prelaminar region of the optic nerve head was noted for TNF-α receptor-1 in the control optic nerve head. (A). Immunostaining was mostly perivascular (v). The intensity of the immunostaining and the number of stained glial cells were greater in the optic nerve heads from patients with primary open angle glaucoma (B) or normal pressure glaucoma (C). Nerve bundles in the prelaminar region also exhibited some immunostaining. (gc, glial column; nb, nerve bundle) (Chromagen, DAB; Nuclear counterstain with Mayer's hematoxylin; original magnification X 100).

Figure 3. Immunoperoxidase staining for TNF-α receptor-1 in the retina of an eye with normal pressure glaucoma. Arrows indicate two retinal ganglion cells exhibiting prominent immunostaining for TNF-α receptor-1 (gc, ganglion cells layer; in, inner nuclear layer; on, outer nuclear layer) (Chromagen,

DAB; Nuclear counterstain with Mayer's hematoxylin; original magnification X 250).

Figure 4. After exposure of co-cultures to stress conditions, apoptosis rate increased in retinal ganglion cells in a time-dependent manner.

Figure 5. Examination of caspase-8 activation using western blot analysis in co-cultured retinal ganglion cells and glial cells. Western blots revealed that after exposure to stress condition, 55-kD immunoreactive band corresponding to caspase-8 cleaved to approximately 30-kD and 20-kD products in retinal ganglion cells. Column 1, retinal ganglion cells incubated under normal condition; column 2, retinal ganglion cells incubated under simulated ischemia; column 3, retinal ganglion cells incubated under elevated pressure; column 4, glial cells incubated under normal condition; column 5, glial cells incubated under simulated ischemia; column 6, glial cells incubated under elevated pressure.

Figure 6. After exposure of co-cultures to stress conditions, TNF-α in conditioned medium in a time dependent manner.

Figure 7. Cultured retinal cells. Following retrograde labeling by Fluoro-Gold and selection of retinal ganglion cells using an immunomagnetic separation method, the selected cells were immunolabeled using antibodies against Fluoro-Gold and Thy-1.1, and examined using flow cytometry. (a) immunolabeling using Fluoro-Gold (FL1-H) and Thy-1.1 (FL3-H) antibodies was co-localized in more than 90% of these cells, while more than 95% of these cells were positive for Thy-1.1. (b) Unselected cells were negative for both Fluoro-Gold (FL1-H) and Thy-1.1 (FL3-H). Cultured retinal ganglion cells had round or oval cell bodies with a diameter of 10-20 □m, phase-bright appearance and branched neuritis of uniform caliber and varying length. (c) A retinal ganglion cell derived from newborn rat retina. (d) Fluorescence microscope image of the retinal ganglion cell shown in panel (c) after labeling

for neurofilament protein. (e) Fluorescence microscope image of the retinal ganglion cell shown in panel (c) after labeling for Thy-1.1. (f) Glial cells derived from newborn rat retina. (g) Fluorescence microscope image of the retinal glial cells shown in panel (i) after labeling for glial fibrillary acidic protein. (h) Fluorescence microscope image of the retinal glial cells shown in panel (i) after labeling for S-100 Magnification bar; c through e, 20 □m; f through h, 60 μm.

Figure 8. Morphologic analysis of apoptotic cell death in co-cultures of retinal ganglion cells and glial cells. Phase contrast microscope image of retinal ganglion cells incubated under normal condition (a), simulated ischemia (b) or elevated hydrostatic pressure (c) for 72 hours. Fluorescence microscope images of TUNEL in panels (d), (e) and (f) correspond to retinal ganglion cells seen in panels (a), (b) and (c), respectively. Phase contrast microscope image of glial cells incubated under normal condition (g), simulated ischemia (h) or under elevated hydrostatic pressure (i) for 72 hours. Fluorescence microscope images of TUNEL in panels (j), (k) and (l) correspond to glial cells seen in panels (g), (h) and (l), respectively. Following incubation of co-cultures under stress conditions, apoptosis was induced in retinal ganglion cells while there was no evidence of apoptosis in glial cells.

Figure 9. (a) Quantitative analysis of positive TUNEL in retinal ganglion cells in co-cultures incubated under simulated ischemia or elevated hydrostatic pressure. (b) Quantitative analysis of positive TUNEL in retinal ganglion cells following passive transfer experiments. Conditioned medium of glial cells cultured alone was collected following their incubation in the presence or absence of simulated ischemia or elevated hydrostatic pressure for 72 hours. Retinal ganglion cells cultured alone were then incubated with the glial conditioned medium for 24 hours.

Figure 10. Examination of caspase activity in co-cultures incubated under simulated ischemia or elevated hydrostatic pressure. (a) Western blot analysis of

caspase-8 expression in co-cultures. (b) Western blot analysis of caspase-3 expression co-cultures. Column 1, control retinal ganglion cells; column 2, retinal ganglion cells incubated under simulated ischemia for 72 hours; column 3, retinal ganglion cells incubated under elevated hydrostatic pressure for 72 hours; column 4, control glial cells; column 5, glial cells incubated under simulated ischemia for 72 hours; olumn 6, glial cells incubated under elevated hydrostatic pressure for 72 hours. Western blots revealed that 55-kD immunoreactive band corresponding to caspase-8 cleaved to 30-kD and 20-kD products in retinal ganglion cells incubated under stress conditions. In addition, 32-kD pro-enzyme caspase-3 cleaved to a 17-kD active subunit in retinal ganglion cells. No cleavage of caspase-8 or caspase-3 was detected using the extracts of glial cells. Caspase activation was also examined, *in Situ*, using Phiphilux-G_6D_2. (c) Retinal ganglion cells incubated under normal condition (d), simulated ischemia (e) or elevated hydrostatic pressure (f) for 72 hours. Fluorescence microscope images seen in panels (f), (g) and (h) correspond to phase contrast images of the retinal ganglion cells seen in panels (c), (d) and (e), respectively. Rhodamine fluorescence (red) indicates caspase-3-like activity in retinal ganglion cells incubated under stress conditions.

Figure 11. Examination of TNF-α and iNOS expression in co-cultures incubated under simulated ischemia or elevated hydrostatic pressure. Both western blot analysis (a and b) and immunocytochemistry (c through h) revealed increased expression of TNF-α and iNOS in glial cells and not in retinal ganglion cells in co-cultures incubated under stress conditions. (a) Western blot analysis of TNF-□ expression. (b) Western blot analysis of iNOS expression. Column 1, control retinal ganglion cells; column 2, retinal ganglion cells incubated under simulated ischemia for 72 hours; column 3, retinal ganglion cells incubated under elevated hydrostatic pressure for 72 hours; column 4, control glial cells; column 5, glial cells incubated under simulated ischemia for 72 hours; column 6, glial cells incubated under elevated hydrostatic pressure for 72 hours. TNF-α expression in glial cells incubated under normal condition (c), under

simulated ischemia for 72 hours (**d**) or under elevated hydrostatic pressure for 72 hours (e). iNOS expression in glial cells incubated under normal condition (f), under simulated ischemia for 72 hours (g) or under elevated hydrostatic pressure for 72 hours (h).

Figure 12. Measurement of TNF-α and end products of NO in conditioned medium of co-cultures incubated under stress conditions. (a) Titers of TNF-α in conditioned medium as measured by ELISA. (b) Titers of end products of NO in conditioned medium as measured by a colorimetric assay.

Figure 13. Inhibition of *apoptosis* in retinal ganglion cells in co-cultures incubated under stress conditions in the presence of specific inhibitors of TNF-α or iNOS. The activity of TNF-□ was neutralized using a specific antibody (10 μg/ml) and iNOS was inhibited using a selective inhibitor, 1400W (2.5 μ M).

DETAILED DESCRIPTION OF THE INVENTION

Although glial cells in the optic nerve head undergo an activation process in glaucoma, the role of glial cells during glaucomatous neurodegeneration of retinal ganglion cells is unknown. Using a co-culture system, the influences of glial cells on survival of retinal ganglion cells following exposure to different stress conditions typified by simulated ischemia and elevated hydrostatic pressure was studied. Following exposure to these stressors, it was observed that glial cells secreted TNF-α as well as other noxious agents such as nitric oxide into the co-culture media and facilitated apoptotic death of retinal ganglion cells as assessed by morphology, TUNEL and caspase activity. The glial origin of these noxious effects was confirmed by passive transfer experiments. Furthermore, retinal ganglion cell apoptosis was attenuated approximately 66% by a neutralizing antibody against TNF-α and 50% by a selective inhibitor (1400W) of inducible nitric oxide synthase. Since elevated intraocular pressure and ischemia are two prominent stress factors identified in the eyes of patients with glaucoma, these findings reveal a novel pathogenic mechanism for retinal ganglion cell death in glaucoma. In

addition, these studies show that inhibition or neutralization of TNF-α released by activated glial cells may provide a novel therapeutic target for neuroprotection in the treatment of glaucomatous optic neuropathy. This invention provides evidence that elevated hydrostatic pressure as well as simulated ischemia can initiate the apoptotic cell death cascade in retinal ganglion cells largely due to the activity of glial cells in response to these stressors. Apoptosis-promoting substances, including TNF-α secreted by activated glial cells after exposure to stress, contribute directly to neuronal cytotoxicity. Further, as shown herein, increased expression of TNF–α and its receptor in the glaucomatous optic nerve head and retina demonstrate a role of this cytokine in the neurodegenerative process of glaucoma, which provides a novel therapeutic target for the management of glaucoma.

This invention provides a method for treating a subject with glaucoma comprising the steps of administrating an agent, compound, or molecule or a composition containing an agent, compound, or molecule, including analogs, isomers, homologues, fragments or variants thereof, which antagonizes, inhibits, inactivates, reduces, suppresses, antagonizes, and/or limits the release, synthesis, or production from cells of TNF-α thereby treating the subject with glaucoma.

In one embodiment, the agent, compound, or molecule suppresses the level or production of TNF-α. In another embodiment, the agent, compound, or molecule inhibits the production of TNF-α.

In another embodiment, the agent, compound, or molecule limits the synthesis or release of TNF-α from cells. In another embodiment, the compound is thalidomide. In another embodiment, the compound is a selective cytokine inhibitor. In another embodiment, the inhibitor is rolipram or phosphodiesterase 4 inhibitor.

In another embodiment, the agent, compound, or molecule inactivates circulating TNF-α. In another embodiment, the molecule is anti- TNF-α antibody. In another embodiment, the molecule is infliximab. In another embodiment, the molecule is

recombinant TNF-α soluble receptors. In another embodiment, the molecule is etanercept.

In another embodiment, the compound or composition contains a molecule which inactivated circulating TNF. In another embodiment, the molecule is anti-TNF antibody. In another embodiment, the molecule is infliximab. In another embodiment, the molecule is recombinant TNF soluble receptors. In another embodiment, the molecule is etanercept. In one embodiment, a selective inhibitor of inducible nitric oxide synthase is provided in combination with the molecules which inactivated circulating TNF or TNF–α reducer.

This invention provides a TNF–α reducer which in one embodiment is hydrazine sulfate, pentoxifylline, ketotifen, tenidap, vesnarinone, cyclosporine, peptide T, sulfasalazine, thorazine, antioxidants, corticosteroids, marijuana, glycyrrhizin, sho-saiko-to, L-carnitine, hyperthermia, or hyperbaric oxygen therapy.

The experiments herein, provide evidence that the functional state of glial cells determined by environmental factors may be important for determining the ultimate role of glial cells as either neuroprotective or neurotoxic. The retinal glial cells exposed to stress conditions such as elevated hydrostatic pressure or simulated ischemia have neurotoxic influence on retinal ganglion cells. Alterations in the functional state of glial cells in response to stress conditions similar to that created during the process of glaucoma, lead to retinal ganglion cell death due to increased production of death-promoting substances, including TNF-α. These findings reveal a novel pathogenic mechanism for retinal ganglion cell death in glaucoma and provide a novel therapeutic target for neuroprotection in the treatment of glaucomatous optic neuropathy.

In the current study, cell survival was examined in primary co-cultures of retinal ganglion cells and glial cells exposed to elevated hydrostatic pressure for a longer period (up to 72 hours) and it was demonstrated that the elevated hydrostatic pressure decreased neuronal survival. Increased production of apoptosis promoting substances by retinal

glial cells following exposure to elevated hydrostatic pressure or simulated ischemia, accounts, in part, for the increased rate of cell death in co-cultured retinal ganglion cells. Passive transfer experiments confirmed that the source of noxious insults on retinal ganglion cells was retinal glial cells exposed to stress conditions.

In addition to TNF-α, as shown herein increased production of NO in retinal glial cells exposed to different stress conditions induced cell death in co-cultured retinal ganglion cells. The experiments herein using inhibitors of TNF-α or iNOS revealed an inhibition of apoptotic cell death in retinal ganglion cells in co-cultures exposed to simulated ischemia or elevated hydrostatic pressure. While iNOS inhibition provided partial protection against apoptotic cell death in co-cultures, more prominent inhibition of apoptosis was observed following inhibition of TNF-α. These results demonstrate a crucial role for endogenous TNF-α in mediating neurotoxicity in cultured retinal ganglion cells. Since TNF-α induces NO secretion, inhibition of its activity thus indirectly decrease the harmful effect created by NO as well. Similar to the observations herein, neutralizing anti-TNF-α antiserum, rather than a NOS inhibitor, inhibited neurotoxicity of cytokine-induced production of iNOS and TNF-α in neuron-astrocyte cultures derived from human fetal cerebrum.

Neutralization of systemic TNF-α ameliorates target organ damage in these diseases. Two drugs which effectively neutralize the adverse effects of TNF-α in rheumatoid diseases are Remicade (Centocor, Malvern, PA), a chimeric monoclonal antibody to TNF-α (Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M, Scallon B, Moore MA, Vilcek J, Daddona P, Ghrayeb J. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol Immunol.* 1993;30:1443-1453; Elliot MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, Woody JN. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet.* 1994;344:1105-1110; Targan SR, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's

disease. Crohn's Disease cA2 Study Group..*N Engl J Med.* 1997;337:1029-1035.) and Enbrel (Immunex, Seattle, WA), a biologically engineered copy of TNF–α receptor p75-Fc Fusion protein (Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med.* 1999;340:253-259.).

This indicates a significant role of TNF–α in the neurodegenerative process seen in glaucoma for drugs inhibiting its function are an attractive targets to decrease cell death in glaucoma. In addition, since TNF–α is a stimulator of nitric oxide synthesis (Romero LI, Tatro JB, Field JA, Reichlin S. Roles of IL-1 and TNF-alpha in endotoxin-induced activation of nitric oxide synthase in cultured rat brain cells. *Am J Physiol.* 1996;270:R326-332; Goureau O, Amiot F, Dautry F, Courtois Y. Control of nitric oxide production by endogenous TNF-alpha in mouse retinal pigmented epithelial and Muller glial cells. *Biochem Biophys Res Commun.* 1997;240:132-135; Heneka MT, Loschmann PA, Gleichmann M, Weller M, Schulz JB, Wullner U, Klockgether T. Induction of nitric oxide synthase and nitric oxide-mediated apoptosis in neuronal PC12 cells after stimulation with tumor necrosis factor-alpha/lipopolysaccharide. *J Neurochem.* 1998;71:88-94.), treatment with TNF–α antagonists, inhibitors, inactivators, reducers, supressors, or agents which antagonize, and/or limits the release, synthesis, or production from cells of TNF-α reduce nitric oxide synthase-2 expression and activity (Perkins DJ, St Clair EW, Misukonis MA, Weinberg JB. Reduction of NOS2 overexpression in rheumatoid arthritis patients treated with anti-tumor necrosis factor monoclonal antibody (cA2). *Arth Rheum.* 1998;41:2205-2210.). Therefore, blockade, amelioration or attentuation of TNF-α is also effective on inhibiting, reducing or preventing nitric oxide synthase-related cell death which is known as an important mediator of neuronal cell death, and may be a causal factor in glaucoma. (Neufeld AH, Sawada A, Becker B. Inhibition of nitric-oxide synthase 2 by aminoguanidine provides neuroprotection of retinal ganglion cells in a rat model of chronic glaucoma Proc Natl Acad Sci U S A 1999 Aug 17;96(17):9944-8)

The type of glaucoma for which the invention is applicable includes but is not

limited to: primary open angle glaucoma, normal pressure glaucoma, pigmentary glaucoma, pseudoexfoliation glaucoma, acute angle closesure glaucoma, absolute glaucoma chronic glaucoma, congenital glaucoma, juvenile glaucoma, narrow angle glaucoma, chronic open angle glaucoma and simplex glaucoma.

As provided herein, the Tumor Necrosis Factor (TNF) superfamily of cytokines includes both soluble and membrane-bound proteins that regulate cellular activation (including immune responses and inflammatory reactions), cellular viability and proliferation, NF-kappa B activation, and also the pathology of various diseases. TNF–α is a cytokine produced by macrophages and lymphocytes which mediates inflammatory and immunopathological responses. TNF–α has been implicated in the progression of diseases which include but are not limited to immunomodulation disorder, infection, cell proliferation, angiogenesis (neovascularisation), tumour metastasis, apoptosis, sepsis, and endotoxaemia. The necrotising action of TNF in vivo mainly relates to capillary injury. TNF causes necrosis not only in tumour tissue but also in granulation tissue. It causes morphological changes in growth inhibition of and cytoxicity against cultured vascular endothelial cells (Haranka et al 1987 Ciba Found Symp 131: 140-153).

Expression of TNF receptors on both lymphoid and non-lymphoid cells can be influenced experimentally by many different agents, such as bacterial lipopolysaccharide (LIPS), phorbol myristate acetate (PMA; a protein kinase C activator), interleukin-1 (IL-1), interferon-gamma (IFN-γ) and IL-2 (Gatanaga et al. Cell Immuno/. 138:1-10, 1991; Yui et al. Placenta 15:819-835, 1994). It has been shown that complexes of human TNF bound to its receptor are internalized from the cell membrane, and then the receptor is either degraded or recycled (Armitage, Curr. Opin. Immunol. 6:407-413, 1994). TNF receptor activity can be modulated using peptides that bind intracellularly to the receptor, or which bind to the ligand binding site, or that affect receptor shedding. See for example patent publications WO 95/31544, WO 95/33051, WO 96/01642, and EP 568 925.

TNF binding proteins (TNF-BP) have been identified at elevated levels in the serum and urine of febrile patients, patients with renal failure, and cancer patients, and even certain

healthy individuals. Human brain and ovarian tumors produced high serum levels of TNF-BP These molecules have been purified, characterized, and cloned (Gatanaga et al., Lymphokine Res. 9:225-229, 1990a; Gatanaga et al., Proc. Nat/. Acad. Sci USA 87:8781-8784, 1990b). Human TNF-BP consists of 30 kDa and 40 kDa proteins which are identical to the N- terminal extracellular domains of p55 and p75 TNF receptors, respectively (US Patent No. 5,395,760; EP 418,014). Such proteins have been suggested for use in treating endotoxic shock. Mohler et al. J. Immunol. 151:1548-1561, 1993 There are several mechanisms possible for the production of secreted proteins resembling membrane bound receptors. One involves translation from alternatively spliced mRNAs lacking transmembrane and cytoplasmic regions.

A "TNF modulator" is a compound that has the property of either increasing or decreasing TNF activity for processing TNF on the surface of cells.

Etanercept (Brand name Embrel) is known to those skilled in the art. Etanercept is a recombinant form of the human tumor necrosis factor receptor fused to the Fc fragment of a human IgG1 molecule. The resulting form is a dimeric molecule that can bind two circulating tumor necrosis factor (TNF) molecules. This binding prevents TNF from interacting with the cell surface TNF receptors, inhibiting its role in the joint pathology. Currently there are two TNF receptors that have been identified (p75 and p55) and both have the same affinity for TNF. Etanercept is supplied in a carton containing four dose trays; each tray contains one 25 mg single-use vial of etanercept, one syringe (1 mL Sterile Bacteriostatic Water for Injection, USP containing 0.9% benzyl alcohol), one plunger, and 2 alcohol swabs. The recommended dose of etanercept for adult patients is 25 mg given twice weekly as a subcutaneous injection.

Infliximab (Remicade) is known to those skilled in the art. Infliximab (Remicade) is a chimeric $IgG1_K$ monoclonal antibody produced by a recombinant cell line to treat Crohn's disease. Infliximab (Remicade) acts by neutralizing the biological activity of TNFþ by high-affinity binding to its soluble and transmembrane forms and inhibits TNFþ receptor binding.

Homologue means a polypeptides having the same or conserved residues at a corresponding position in their primary, secondary or tertiary structure. The term also extends to two or more nucleotide sequences encoding the homologous polypeptides.

A "nucleic acid" or "polynucleotide" refers to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules") in either single stranded form, or a double-stranded helix. Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA" is a DNA that has undergone a molecular biological manipulation.

"Substantial identity" or "substantial sequence identity" mean that two sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap which share at least 65-99 percent sequence identity, share at least 75 percent sequence identity, share at least 80 percent sequence identity, share at least 90 percent sequence identity, preferably at least 95 percent sequence identity, more preferably at least 99 percent sequence identity or more. The following terms are used to describe the sequence relationships between two or more nucleic acid molecules or polynucleotides: "reference sequence", "comparison window", "sequence identity", "percentage of sequence identity", and "substantial identity". A "reference sequence" is a defined sequence used as a basis for a sequence comparison; a reference sequence may be a subset of a larger sequence, for example, as a segment of a full-length cDNA or gene sequence given in a sequence listing or may comprise a complete cDNA or gene sequence.

Optimal alignment of sequences for aligning a comparison window may be conducted by the local homology algorithm of Smith and Waterman (1981) *Adv. Appl. Math.* 2:482, by the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. (USA)* 85:2444, or by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Dr., Madison, WI).

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation, such as conjugation with a labeling component.

A "fusion polypeptide" is a polypeptide comprising regions in a different position in the sequence than occurs in nature. The regions can normally exist in separate proteins and are brought together in the fusion polypeptide; they can normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide; or they can be synthetically arranged. A "functionally equivalent fragment" of a polypeptide varies from the native sequence by addition, deletion, or substitution of amino acid residues, or any combination thereof, while preserving a functional property of the fragment relevant to the context in which it is being used. Fusion peptides and functionally equivalent fragments are included in the definition of polypeptides used in this disclosure.

It is understood that the folding and the biological function of proteins can accommodate insertions, deletions, and substitutions in the amino acid sequence. Some amino acid substitutions are more easily tolerated. For example, substitution of an amino acid with hydrophobic side chains, aromatic side chains, polar side chains, side chains with a positive or negative charge, or side chains comprising two or fewer carbon atoms, by another amino acid with a side chain of like properties can occur without disturbing the

essential identity of the two sequences. Methods for determining homologous regions and scoring the degree of homology are described in Altschul et al. Bull. Math. Bio. 48:603- 616, 1986; and Henikoff et al. Proc. Natl. Acad. Sci. USA 89:10915-10919, 1992.

Substitutions that preserve the functionality of the polypeptide, or confer a new and beneficial property (such as enhanced activity, stability, or decreased immunogenicity) are especially preferred.

An "antibody" (interchangeably used in plural form) is an immunoglobulin molecule capable of specific binding to a target, such as a polypeptide, through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact antibodies, but also antibody equivalents that include at least one antigen combining site of the desired specificity. These include but are not limited to enzymatic or recombinantly produced fragments antibody, fusion proteins, humanized antibodies, single chain variable regions, diabodies, and antibody chains that undergo antigen-induced assembly. In one embodiment the antibody is a monoclonal antibody. In another embodiment the antibody is a polyclonal antibody. The antibody may be chimeric, human or murine or a hybrid thereof which are known to those skilled in the art. Specifically binds to an "antibody" or "specifically immunoreactive with", when referring to the recombinant antibody or proteins refers to the binding of a cell or protein to the TNF so as to modulate, decrease, suppress, inavtivate the activity of TNF.

Polyclonal antibodies against these peptides may be produced by immunizing animals using the selected peptides. Monoclonal antibodies are prepared using hybridoma technology by fusing antibody producing B cells from immunized animals with myeloma cells and selecting the resulting hybridoma cell line producing the desired antibody. Alternatively, monoclonal antibodies may be produced by in vitro techniques known to a person of ordinary skill in the art. These antibodies are useful to detect the expression of polypeptide encoded by the isolated DNA molecule of the DNA virus in living animals, in humans, or in biological tissues or fluids isolated from animals or

humans.

Antibodies Polyclonal antibodies can be prepared by injecting a vertebrate with a polypeptide of this invention in an immunogenic form. Immunogenicity of a polypeptide can be enhanced by linking to a carrier such as KLH, or combining with an adjuvant, such as Freund's adjuvant. Typically, a priming injection is followed by a booster injection is after about 4 weeks, and antiserum is harvested a week later. Unwanted activity cross-reacting with other antigens, if present, can be removed, for example, by running the preparation over adsorbants made of those antigens attached to a solid phase, and collecting the unbound fraction. If desired, the specific antibody activity can be further purified by a combination of techniques, which may include protein, A chromatography, ammonium sulfate precipitation, ion exchange chromatography, HPLC, and immunoaffinity chromatography using the immunizing polypeptide coupled to a solid support. Antibody fragments and other derivatives can be prepared by standard immunochemical methods, such as subjecting the antibody to cleavage with enzymes such as papain or pepsin.

The antibody may be labeled with a detectable marker including, but not limited to: a radioactive label, or a colorimetric, a luminescent, or a fluorescent marker, or gold. Radioactive labels include, but are not limited to: ^{3}H, ^{14}C, ^{32}P, ^{33}P; ^{35}S, ^{36}Cl, ^{51}Cr, ^{57}Co, ^{59}Co, ^{59}Fe, ^{90}Y, ^{125}I, ^{131}I, and ^{186}Re. Fluorescent markers include but are not limited to: fluorescein, rhodamine and auramine. Colorimetric markers include, but are not limited to: biotin, and digoxigenin. Examples of types of labels encompassed by the present invention include, but are not limited to, radioisotopic labels (e.g., .sup.3 H, .sup.125 I, .sup.131 I, .sup.35 S, .sup.14 C, etc.), non-radioactive isotopic labels (e.g., .sup.55 Mn, .sup.56 Fe, etc.), fluorescent labels (e.g., a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, art O-phthaldehyde label, a fluorescamine label, etc.) for example, as in peridinin chlorophyll protein (PerCP), chemiluminescent labels, enzyme labels (e.g., alkaline phosphatase, horse radish peroxidase, etc.), protein labels, labels useful in radioimaging and radioimmunoimaging.

Variant(s), as the term is used herein, are polynucleotides or polypeptides that differ from a reference polymicleotide or polypeptide respectively. Variants in this sense are described below and elsewhere in the present disclosure in greater detail. (1) A polynucleotide that differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may be silent, i.e., they may not alter the amino acids encoded by the polynucleotide. Where alterations are limited to silent changes of this type a variant will encode a polypeptide with the same amino acid sequence as the reference polypeptide. Changes in the nucleotide sequence of the variant may alter the amino acid sequence of a polypeptide encoded by the reference polymicleotide. Such nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. (2) A polypeptide that differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference and the variant are closely similar overall and, in many region, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions, fusions and truncations, which may be present in any combination. (3) A variant may also be a fragment of a polynucleotide or polypeptide of the invention that differs from a reference polynucleotide or polypeptide sequence by being shorter than the reference sequence, such as by a terminal or internal deletion. A variant of a polypeptide of the invention also includes a polypeptide which retains essentially the same biological function or activity as such polypeptide, e.g., proproteins which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide. (4) A variant may also be (i) one in which one or more of the amino acid residues are substituted with a conserved or non- conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. (5) A variant of the polynucleotide or polypeptide may be a

naturally occurring variant such as a naturally occurring allelic variant, or it may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the polynucleotide may be made by mutagenesis techniques, including those applied to polynucleotides, cells or organisms, or may be made by recombinant means. Among polynucleotide variants in this regard are variants that differ from the aforementioned polynucleotides by nucleotide substitutions, deletions or additions. The substitutions, deletions or additions may involve one or more nucleotides. The variants may be altered in coding or non- coding regions or both. Alterations in the coding regions may produce conservative or non- conservative amino acid substitutions, deletions or additions. All such variants defined above are deemed to be within the scope of those skilled in the art from the teachings herein and from the art.

Antisense nucleotides or polynucleotide sequences are useful in preventing or diminishing the expression of TNF are known to those skilled in the art. Also, this invention provides an antisense molecule capable of specifically hybridizing with TNFα to inhibit or repress production of TNFα. This invention provides an antagonist capable of blocking the expression of TNF. In one embodiment the antagonist is capable of hybridizing with a double stranded DNA molecule. In another embodiment the antagonist is a triplex oligonucleotide capable of hybridizing to the DNA molecule. In another embodiment the triplex oligonucleotide is capable of binding to at least a portion of TNF.

The antisense molecule may be DNA or RNA or variants thereof (i.e. DNA or RNA with a protein backbone). The present invention extends to the preparation of antisense nucleotides and ribozymes that may be used to interfere with the expression of the receptor recognition proteins at the translation of a specific mRNA, either by masking that MRNA with an antisense nucleic acid or cleaving it with a ribozyme.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific MRNA molecule. In the cell, they hybridize to that MRNA, forming a double stranded molecule. The cell does not translate an MRNA in this double-stranded form. Therefore, antisense nucleic acids interfere with the expression

of MRNA into protein.

Oligonucleotides which are complementary to TNF and which may bind to TNF and inhibit production of TNF may be obtained as follows: The polymerase chain reaction is then carried out using the two primers. See *PCR Protocols: A Guide to Methods and Applications* [74]. Following PCR amplification, the PCR-amplified regions of a viral DNA can be tested for their ability to hybridize to the three specific nucleic acid probes listed above. Alternatively, hybridization of a viral DNA to the above nucleic acid probes can be performed by a Southern blot procedure without viral DNA amplification and under stringent hybridization conditions as described herein. High stringent hybridization conditions are selected at about 5 C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 60C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents, ie. salt or formamide concentration, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one. For Example high stringency may be attained for example by overnight hybridization at about 68C in a 6x SSC solution, washing at room temperature with 6x SSC solution, followed by washing at about 68 C in a 6x SSC in a 0.6x SSX solution.

Hybridization with moderate stringency may be attained for example by: 1) filter pre-hybridizing and hybridizing with a solution of 3x sodium chloride, sodium citrate (SSC), 50% formamide, 0.1M Tris buffer at Ph 7.5, 5x Denhardt's solution; 2.) pre-hybridization at 37 C for 4 hours; 3) hybridization at 37 C with amount of labelled probe equal to 3,000,000 cpm total for 16 hours; 4) wash in 2x SSC and 0.1% SDS solution; 5) wash 4x for 1 minute each at room temperature at 4x at 60 C for 30 minutes each; and 6) dry and expose to film.

The phrase "selectively hybridizing to" refers to a nucleic acid probe that hybridizes, duplexes or binds only to a particular target DNA or RNA sequence when the target sequences are present in a preparation of total cellular DNA or RNA. By selectively hybridizing it is meant that a probe binds to a given target in a manner that is detectable in a different manner from non-target sequence under high stringency conditions of hybridization. in a different "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid probe. Proper annealing conditions depend, for example, upon a probe's length, base composition, and the number of mismatches and their position on the probe, and must often be determined empirically. For discussions of nucleic acid probe design and annealing conditions, see, for example, Sambrook *et al.*, [81] or Ausubel, F., *et al.*, [8].

As used herein, "pharmaceutical composition" means therapeutically effective amounts of the compound or composition containing the molecule of the invention as described above together with suitable diluents, preservatives, solubilizers, emulsifiers, adjuvant and/or carriers. A "therapeutically effective amount" as used herein refers to that amount which provides a therapeutic effect for a given condition and administration regimen. Such compositions are liquids or lyophilized or otherwise dried formulations and include diluents of various buffer content (e.g., Tris-HCl., acetate, phosphate), pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts). solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), bulking substances or tonicity modifiers (e.g., lactose, mannitol), covalent attachment of polymers such as polyethylene glycol to the protein, complexation with metal ions, or incorporation of the material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. In one embodiment the pharmaceutical composition is administered parenterally,

intratumorally, paracancerally, transmucosally, transdermally, intramuscularly, intravenously, intradermally, intravascularly, subcutaneously, intraperitonealy, intraventricularly, intracranially, topical drops or ointment, periocular injection, systemically by intravenous injection or orally, intracamerally into the anterior chamber or vitreous, via a depot attached to the intraocular lens implant inserted during surgery, or via a depot placed in the eye sutured in the anterior chamber or vitreous.

Further, as used herein "pharmaceutically acceptable carrier" are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobial, antioxidants, collating agents, inert gases and the like.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. An adjuvant can serve as a tissue depot that slowly releases the antigen and also as a lymphoid system activator that non-specifically enhances the immune response (Hood et al., *Immunology, Second Ed.*, 1984, Benjamin/Cummings: Menlo Park, California, p. 384). Often, a primary challenge with an antigen alone, in the absence of an adjuvant, will fail to elicit a humoral or cellular immune response. Adjuvant include, but are not limited to, complete Freud's adjuvant, incomplete Freud's adjuvant, saponin, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil or hydrocarbon emulsions, keyhole limpet hemocyanins, dinitrophenol. Preferably, the adjuvant is pharmaceutically acceptable.

Controlled or sustained release compositions include formulation in lipophilic depots (e.g. fatty acids, waxes, oils). Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors. Other embodiments of the compositions of the invention incorporate particulate forms protective coatings, protease inhibitors or permeation enhancers for various routes of administration, including parenteral, pulmonary, nasal and oral. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents which enhance the effectiveness of the active ingredient.

An active component can be formulated into the therapeutic composition as neutralized pharmaceutically acceptable salt forms. Pharmaceutically acceptable salts include the acid addition salts and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed from the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The pharmaceutically acceptable form of the composition includes a pharmaceutically acceptable carrier. In the therapeutic methods and compositions of the invention, a therapeutically effective dosage of the active component is provided. A worker based on patient characteristics (age, weight, sex, condition, complications, other diseases, etc.), as is well known in the art. Furthermore, as further routine studies are conducted, more specific information will emerge regarding appropriate dosage levels for treatment of various conditions in various patients, and the ordinary skilled worker, considering the therapeutic context, age and general health of the recipient, is able to ascertain proper dosing. Generally, for intravenous injection or infusion, dosage may be lower than for

intraperitoneal, intramuscular, or other route of administration. The dosing schedule may vary, depending on the circulation half-life, and the formulation used. The compositions are administered in a manner compatible with the dosage formulation in the therapeutically effective amount. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual. However, suitable dosages may range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably one to several, milligrams of active ingredient per kilogram body weight of individual per day and depend on the route of administration. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed by repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous. infusion sufficient to maintain concentrations of ten nanomolar to ten micromolar in the blood are contemplated.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.